EYE FACET NUMBER AS INFLUENCED BY TEMPERATURE IN THE BAR-EYED MUTANT OF DROSOPHILA MELANOGASTER (AMPELOPHILA).[1]

E. W. SEYSTER,
UNIVERSITY OF ILLINOIS.

The effect of temperature upon facet number in the red bar-eyed *Drosophila* was studied in connection with an extended series of experiments on the effect of selection which is in progress in the Zoölogical Laboratory at the University of Illinois.

For the higher temperatures (27°–30°) a bacteriological incubator, varying not more than one degree centigrade was used. The room temperature controlled by a Johnson regulator, recorded by means of a thermograph, and varying not more than 2°, was used for medium temperatures. A cold plate arranged by allowing tap water to flow around a dish in which the *Drosophila* bottles were placed and varying not more than 1° during any one experiment, was used for the low temperature.

The parents in each experiment were taken from mass cultures or stocks, no attempt being made to obtain virgin females for this work. However, the average facet number of the parents of any two sets in an experiment differed but little, any difference occurring being much less than the difference between the averages of the offspring raised at different temperatures. In experiments 14, 15, 17, and 18 the parents were left in the bottles for one day only; in all other experiments they were allowed to remain for nearly the full developmental period in order that a larger number of individuals might be obtained.

That light is not an important factor for facet number was determined by control experiments.

[1] Contribution from the Zoölogical Laboratory of the University of Illinois, no. 141.

The manuscript of Mr. Seyster's paper essentially in its present form was completed in May, 1917, when he entered the U. S. Army Training Camp. At that time it was hoped that he might eventually follow up the work but those plans have not materialized and it seems best to publish the results as they stand. C. Z.

In counting facets, a no. 4 eye-piece and a no. 3 Leitz objective were used. In some cases the facets were counted after the flies had been preserved in alcohol, but mostly the counts were made shortly after the emergence of the flies from the pupa cases. In order to determine the per cent. of error made in counting, a set of ten flies, five males and five females was counted at two different times. The total number of facets in the first count was 1,347, in the second count 1,356, and the per cent. of difference between the two counts was 0.81 per cent. For a larger number of individuals the per cent. of error would probably be less than this; for single individuals it was found to average about 5 per cent. to 7 per cent.

The area of the eye in arbitrary units was found by making an outline camera lucida drawing of the eye, and measuring the enclosed area with a polar planimeter, which was found to give accurate results to 2/100 of a square inch.

DATA.

Experiments 1, 2, 3 (Tables I. and II.).—These experiments were designed originally to test the effect of different amounts of food on facet number. Three bottles, each containing a different

TABLE I.

EXPERIMENTS 1, 2, 3, MALES.

	Parents.				Offspring.				Difference Between Parents and Offspring.	Stock at End of Experiment.
Temperature.....	(21°–23°)				27°					19–21
Catalog number.....	1	2	3	Total	1	2	3	Total		
Number of individuals.....	11	10	8	29	25	20	21	66		24
Facet averages.....	105	112	106	108	85	86	77	82	26	164
Probable errors.....				±3.0				±1.7		
High variates.....	149	164	126	164	162	178	140	178	14	202
Low variates.....	77	86	84	77	39	45	36	36	41	112

amount of food and thirteen pairs of flies as parents, were placed in a small well-lighted room where the temperature was kept at approximately 27°. After seven days the parents were taken out and their facets counted. It was found that the three bottles differed slightly from one another in facet number but that their

average facet number was decidedly lower than that of the parents. In the male flies this difference was 26, and in the female flies 35. A count of the stock was made at the end of the experiment, and it was found that here the facet number had

TABLE II.

EXPERIMENTS 1, 2, 3. FEMALES.

	Parents.				Offspring.				Difference Between Parents and Offspring.	Stock at End of Experiment.
Temperature.....	(21°–23°)				27°					19–21
Catalog number.....	1	2	3	Total	1	2	3	Total		
Number of individuals.....	13	12	10	35	30	30	18	78		19
Facet averages.....	75	89	82	82	47	54	42	47	35	109
Probable errors.....				±2.2				±1.3		
High variates.....	100	127	98	127	88	102	86	102	25	146
Low variates.....	43	52	65	43	31	30	26	26	17	54

increased, the average for the males being 50 higher than that of the parents, and for the females 30. Previous to the time of the experiment, the stock had been kept in a room where the temperature was approximately 21°–23°, while, during the time of the experiment, it was kept at 19°–21°.

Experiment 4 (Tables III., IV.).—Experiments 1, 2 and 3

TABLE III.

EXPERIMENT 4. MALES.

	Parents.				Offspring.			Differences in Facet Number Among Offspring at Different Temperatures.	
Temperature.....	22°				28.5°	17.5°	22°	17.5°–22.0°	22.0°–28.5°
Catalog number.....	4.1	4.2	4.3	Total	4.1	4.2	4.3		
Number of individuals...	10	13	12	35	80	29	17		
Facet averages.....	135	135	122	131	80	153	121	32	41
Probable errors.....				±3.4	±2.6				
High variates.....	192	174	180	192	161	202	186	16	25
Low variates.....	101	77	82	77	41	120	87	33	46

indicated that temperature is an important factor in facet variation. To test this more fully one set of *Drosophila* was placed in an incubator, where the temperature was 28.5°, another was kept at room temperature, 22°, and a third set was placed on a cold plate at 17.5°. The offspring were then allowed to

develop at these temperatures. In facet number, those which developed at room temperature gave an average of 121 for the males and 88 for the females, and differed but little from the parents; those which developed at 17.5° averaged 153 for the males and 149 for the females; and those which developed at 28.5° averaged 80 for the males and 45 for the females. In the above experiment, the electric current of the incubator was turned off for one and one half days so that the developing flies of the warm set were at room temperature for that length of time. In

TABLE IV.

EXPERIMENT 4. FEMALES.

	Parents.				Offspring.			Differences in Facet Number Among Offspring at Different Temperatures.	
Temperature	22°				28.5°	17.5°	22°	17.5°–22.0°	22.0°–28.5°
Catalog number	4.1	4.2	4.3	Total	4.1	4.2	4.3		
Number of individuals	22	21	24	67	63	18	25		
Facet averages	84	75	76	79	45	149	88	61	43
Probable errors				±1.4			±1.0		
High variates	121	109	113	121	83	188	126	62	43
Low variates	43	57	44	43	20	106	62	44	42

the counts, the effect of this may be noted as about one ninth of the flies is much higher than the rest, the developmental period being nine days at a temperature of 28.5°. The temperature of the cold plate was allowed to rise to that of the room twice for about three hours each time, so that the results are somewhat less striking in this experiment than in some others as regards the difference between high and low facet numbers.

TABLE V.

EXPERIMENT 4. 1. MALES.

	Parents.[1]			Offspring.		Differences Between Parents and Offspring.	
Temperature	28.5°			28.5°	17.0°	28.5°–28.5°	17.0°–28.5°
Catalog number	4.11	4.12	Total	4.11	4.12		
Number of individuals	10	13	23	19	5		
Facet averages	89	72	79	66	135	23	63
High variates	140	120	140	86	148	54	28
Low variates	60	43	43	44	116	16	73

Experiments 4.1 and 4.2 (Tables V., VI., VII., VIII.).—Part of the offspring of experiment 4 which developed at different temperatures, was used as parents in similar experiments, only here flies developing at the room temperature were not used.

TABLE VI.

EXPERIMENT 4. 1. FEMALES.

	Parents.[1]			Offspring.		Differences Between Parents and Offspring.	
Temperature	28.5°			28.5°	17.0°	28.5°–28.5°	17°–28.5°
Catalog number	4.11	4.12	Total	4.11	4.12		
Number of individuals	13	17	30	21	4		
Facet averages	54	46	49	58	115	4	69
High variates	83	76	83	94	122	11	46
Low variates	47	31	31	40	106	7	75

TABLE VII.

EXPERIMENT 4. 2. MALES.

	Parents.			Offspring.		Differences Between Parents and Offspring.	
Temperature	17.5°			28.5°	17.0°	17.5°–28.5°	17.5°–17.0°
Catalog number	4.21	4.22	Total	4.21	4.22		
Number of individuals	13	11	24	31	9		
Facet averages	158	151	155	94	185	64	34
High variates	202	172	202	138	228	64	26
Low variates	128	131	128	50	136	78	5

TABLE VIII.

EXPERIMENT 4. 2. FEMALES.

	Parents.			Offspring.		Differences Between Parents and Offspring	
Temperature	17.5°			28.5°	17.0°	17.0°–28.5°	17.5°–17.0°
Catalog number	4.21	4.22	Total	4.21	4.22		
Number of individuals	13	11	24	31	9		
Facet averages	142	136	139	55	99	87	37
High variates	163	165	165	74	121	89	44
Low variates	108	106	106	28	65	80	41

The results obtained from all four sets are analogous to those obtained from experiment four. The results from the above experiments indicate that the temperature at which the parents

[1] Development at room temperature for one and one half days.

develop has little, if any, effect upon the facet number of the offspring.

Experiment 5 (Tables IX., X.).—This experiment is the most conclusive as a high temperature of 29° and a low temperature

TABLE IX.

EXPERIMENT 5. MALES.

	Parents.			Offspring.		Differences in Facet Number at Different Temperatures.
Temperature.....	22°			29°	17.5°	
Catalog number.....	5.1	5.2	Total	5.1	5.2	
Number of individuals.	10	10	20	86	64	
Facet averages.....	129	127	128	56	167	111
Probable errors.....			±5.6	±0.9	±3.0	
High variates.....	200	211	211	98	226	128
Low variates.....	90	80	80	34	74	40
Coefficient of variation.			25	24	22	

of 17.5° were maintained throughout. The male flies developing at the low temperature showed an average facet number three times as great as that of those developing at the higher temperature, the corresponding ratio for the female flies being 4.7. The average facet number of the male flies developing at 29° was 56, of those developing at 17.5°, 167; the average facet

TABLE X.

EXPERIMENT 5. FEMALES.

	Parents.			Offspring.		Differences in Facet Number at Different Temperatures.
Temperature.....	22°			29°	17.5°	17.5°–29°
Catalog number.....	5.1	5.2	Total	5.1	5.2	
Number of individuals.	12	12	24	71	75	
Facet averages.....	97	87	92	27	128	101
Probable errors.....			±3.0	±0.5	±1.5	
High variates.....	138	144	144	43	188	145
Low variates.....	34	53	34	16	83	67
Coefficient of variation.			25	21	20	

number of the females developing at 29° was 27, of those developing at 17.5°, 128. The parents of the above sets, developing at 22° showed little difference in the average facet number. In the males this difference was 2 facets, in the females it was 10. In both males and females the parents which had the higher facet average gave offspring with the lower average, these being

the ones that developed at 29°. The extreme variates (high, low) of the parents of the two sets are slightly greater in the case of the set which developed at the lower temperature, but as the greater difference here is only eleven facets, and as the difference between the variates of the two sets of offspring is as great as 145, it is certain that selection can play no great rôle in determining the facet difference of the offspring here noted.

Experiment 5.1 (Tables XI., XII.).—Parents of this experiment are the offspring of set 5.1 in experiment 5. In experiment 5.1, one set of offspring developed at 29° while another developed at 17°. The male flies developing at 29° gave an average which was 5 facets less than their parents which developed at the same temperature while the male offspring developing at 17° had a facet average which was 127 greater than that of the parents. The facet average of the female offspring was 11 greater for those developing at 29° and 120 greater for those developing at 17°, than that of the parents which developed at 29°.

TABLE XI.

EXPERIMENT 5. 1. MALES.

	Parents (from 5.1 Offspring).	Offspring.		Difference Between Parents and Offspring.	
Temperature	29°	29°	17°	29°–29°	17°–29°
Catalog number		5.11	5.12		
Number of individuals		7	21		
Facet averages	56	51	183	5	127
High variates	98	61	303	37	205
Low variates	34	38	127	4	93

TABLE XII.

EXPERIMENT 5. 1. FEMALES.

	Parents (from 5.1 Offspring).	Offspring.		Difference Between Parents and Offspring.	
Temperature	29°	29°	17°	29°–29°	17°–29°
Catalog number		5.11	5.12		
Number of individuals		16	26		
Facet averages	27	38	147	11	120
High variates	43	55	235	12	192
Low variates	16	27	111	11	95

A part of the offspring from set 5.11 was continued for three more generations at 29° (Table XIII.). The fifth generation

gave an average of 63 for the males and 35 for the females. The males were lower and the females higher than the first generation (Experiment 5) which developed at 29°.

TABLE XIII.

THREE MORE GENERATIONS AT 29°—A CONTINUATION OF THE 5.11 LINE.

	Males.			Females.		
Generation number	3	4	5	3	4	5
Facet averages	42	26	63	35	44	35
Number of individuals	17	7	55	9	25	52
High variates	58	86	130	46	56	49
Low variates	32	43	31	28	25	20

Experiments 14, 15, 16, 17, 18 (Tables XIV., XV., XVI., XVII., XVIII.).—In experiments 14, 15 and 17, the effect of temperature on the white bar-eye *Drosophila* was determined,

TABLE XIV.

PARENTS. EXPERIMENTS 14, 15, 17. DEVELOPED AT 27° C.

Males.

Catalog Number	14.1 15.3 17.2	14.2 15.4 17.3	14.3 15.5 17.4	14.4 15.6 17.5	14.5 15.1 17.6	14.6 15.2 17.1
Number of individuals	16	14	15	6	12	15
Facet averages	117	115	106	120	119	108
Probable errors ±	4.6	6.0	3.0	6.9	5.9	4.8
High variates	166	206	136	126	161	184
Low variates	56	60	72	82	82	75

Females.

Number of individuals	24	22	19	18	18	23
Facet averages	73	69	77	74	75	75
Probable errors ±	2.6	2.2	2.2	2.7	2.2	2.2
High variates	107	103	116	110	102	102
Low variates	44	44	57	47	38	40

and the results are fully concurrent with the results obtained from the work on the red-eye. Experiment 18 gives additional data for the red bar eye.

These four experiments were made in order to determine the period of development when temperature is most effective. To determine this, five bottles with parents were placed at 29° and the developing flies in the different bottles were allowed to pass different fractions of their developmental period here. They were then removed to the cold plate at 15°, or to the room at

22°, and allowed to complete their development at these temperatures. One bottle was kept on the cold plate throughout the period of development, and another was kept at 29°. Removals

TABLE XV.

EXPERIMENT 14. OFFSPRING. PART OF DEVELOPMENT 29°—REST AT 15°.

Males.

Catalog Number.	14.1.	14.2.	14.3.	14.4.	14.5.	14.6.
Number of days at 29°	0	½	2	4¼	6½	all
Developmental period in days	33	31	28	21½	10	8
Number of individuals	6	9	13	6	37	22
Facet averages	282	272	352	57	64	76
Probable errors ±	13	7.3	9	2	2.4	1.7
High variates	366	325	414	65	130	161
Low variates	240	217	280	52	45	39
Females.						
Number of individuals	10	21	18	10	25	21
Facet averages	215	266[1]	231	56	56	59
Probable errors ±	11	16	5	2.4	1.4	1.1
High variates	288	650[1]	281	84	86	80
Low variates	147	190	171	43	39	48

from 29° to a lower temperature were made as follows: Number 2, after one half day; number 3, when the larvæ were about one third grown (2–2½ days); number 4, just before pupation

TABLE XVI.

EXPERIMENT 15. OFFSPRING. PART OF DEVELOPMENT AT 29°. REST AT 15°.

Males.

Catalog Number	15.1	15.2	15.3	15.4	15.5	15.6
Number of days at 29°	0	½	2½	4½	9	all
Developmental period days				21	11½	9
Number of individuals	4	17	4	2	21	19
Facet averages	304	271	293	76	63	60
High variates	324	402	326	88	88	98
Low variates	290	161	248	65	40	42
Females.						
Number of individuals	2	18	8	2	27	32
Facet averages	172	188	233	41	41	43
High variates	180	233	289	47	66	63
Low variates	164	130	178	36	33	27

had started; number 5, after most of the larvæ had transformed into pupæ. Number 6 was kept at 29°. In experiment 18, removals from room temperature (22°) to 29° were made, and

[1] Average includes female with 650 facets. She may be heterozygous for bar.

instead of using white bar-eyed flies, the red bar-eyed were used. As the parents were kept in the bottles for one day, the length of time the developing flies were allowed to remain at the initial

TABLE XVII.

EXPERIMENT 17. PART OF DEVELOPMENT AT 29°. REST AT 22°.

Males.

Catalog Number	17.1.	17.2.	17.3.	17.4.	17.5.	17.6.
Number of days at 29°	0	½	2½	4¼	7½	all
Developmental period days	12	12	12	11	8½	8½
Number of individuals	31	6	5	45	25	25
Facet averages	131	170	127	102	65	69
Probable errors ±	2.9	7.4	8.1	2.4	3.6	2.3
High variates	171	198	180	150	144	129
Low variates	99	125	96	36	32	42
Females.						
Number of individuals	32	8	4	50	30	29
Facet averages	114	130	101	66	39	44
Probable errors ±	2.4	3.0	5.1	2.3	1.0	1.0
High variates	172	160	117	132	62	61
Low variates	80	112	80	32	25	31

temperature was reckoned from the middle of that day and not from the beginning of the experiment, in order to give the mean time of development at that temperature for all the eggs laid during that day.

TABLE XVIII.

EXPERIMENT 18. PART OF DEVELOPMENT AT 22°. REST AT 29°. RED BAR-EYE.

Males.

Catalog Number.	18.1.	18.2.	18.3.	18.4.	18.5.
Number of days at 22°	0	2½	6½	9½	all
Developmental period days	8¾	9¾	11	11¾	12½
Number of individuals	9	12	11	14	8
Facet averages	89	98	146	175	189
High variates	167	166	164	210	291
Low variates	49	64	65	128	124
Females.					
Number of individuals	4	11	13	19	12
Facet averages	40	45	104	150	130
High variates	47	68	172	183	213
Low variates	35	35	52	120	101

In experiments 14 and 15 the effect of temperature is to be noted early in the larval period; in 17 and 18, which were changed from 29° to 22° and from 22° to 29° respectively, the effect of

temperature is to be noted throughout the larval life. In no case was there any significant effect upon the facet number of the flies after the pupæ had been formed. The parent flies of the six different sets differed but little in facet number. In experiments 14, 15 and 17 each set of parents was used in each experiment. For instance, the parents of 14.1, 15.3 and 17.2 were identical. In experiment 18 the same parents were used throughout, being changed from one bottle to another.

Facet Change per Degree Change in Temperature.—With one exception, the number of facets per degree change in temperature varies from 5.2 to 8.9 and is fairly constant. The following is a list of facets per degree change, found by dividing the difference in facet averages by the difference in degrees centigrade:

Experiment.	Temperatures.	Facet Average Low Temperatures.	Facet Average High Temperatures.	Facet Differences.	Facet Difference. / Temp. Difference.
1, 2, 3, males.....	22° –27°	108	82	26	– 5.2
1, 2, 3, females.....	22° –27°	82	47	35	– 7.0
4 males.....	17.5°–22°	153	121	32	– 7.1
4 females.....	17.5°–22°	149	88	61	–13.5
4 males.....	22° –28.5°	121	80	41	– 6.3
4 females.....	22° –28.5°	88	45	43	– 6.6
5 males.....	17.5°–29°	167	56	111	– 8.9
5 females.....	17.5°–29°	128	27	101	– 8.0

The relation between the facet number and a ten degree difference in temperature is also very interesting. Is the number of facets increased two to three times per ten degrees decrease? The following table gives the results, n_t being the number of facets at the lower temperature and n_{t+10} at the higher temperature:

Experiment.	♀ $\frac{n_t}{n_{t+10}}$.	♂ $\frac{n_t}{n_{t+10}}$.
1, 2, 3	2.6	3.5
4	1.7	3.0
5	2.5	3.9
5.1	3.0	3.2
Average,	2.6	3.5

Size of Individual Facets (Plate I.).—The relation of the area of the eye to facet number was found to be constant for flies with an intermediate number of facets. This constant, for 43 individuals, was found by dividing the facet number by the area of

the camera lucida drawing in 1/100 sq. in. In flies with a very small number of facets the relation does not hold; in very large eyes, the curved surface makes it difficult to obtain accurate areas. Leaving out of consideration these extremes, the change in facet number is accompanied by a corresponding change in the size of the eye, and there is no change in the size of facets.

Discussion.

Observations upon the red and white bar-eye show that temperature is an important factor in determining facet number of the flies taken at random from a general population. Flies raised at a higher temperature, however, do not have a higher number of facets, but on the contrary, the higher the temperature of development, the lower the facet number. An explanation which may be offered presupposes the existence of a chemical factor or determiner which acts as an inhibitor of facet formation and that, at a higher temperature, the speed of reaction is much greater than at a lower. Then, according to Van't Hoff's law, with an increase of 10° C. the speed of the reaction should be from two to three times as great and the number of facets one half to one third times as large, and vice versa. An examination of the data shows a considerable degree of approximation to this condition. In the males with a 10° decrease the facet average is 2.6 times as great; in the females 3.5 times. Whether or not the high value, 3.5, in the females is due to the fact that bar-eye is sex linked and the female receives two chromosomes containing this factor, while the male receives but one, is a matter for speculation.

Summary.

1. Temperature is an important factor in the determination of facet number in the bar-eye of *Drosophila*.

2. A lower developmental temperature results in a higher facet number, and conversely a higher temperature results in a lower facet number.

3. With each 10° drop in temperature between 29° and 15°, the facet number is increased on the average 2.6 times in the males and 3.5 times in the females.

4. The increase in facet number except at the extremes is

directly proportional to the increase in the area of the eye, and facet-size is therefore a constant within these limits.

5. Light, and amount of food, as they occur in these experiments are not important factors in the determination of facet number.

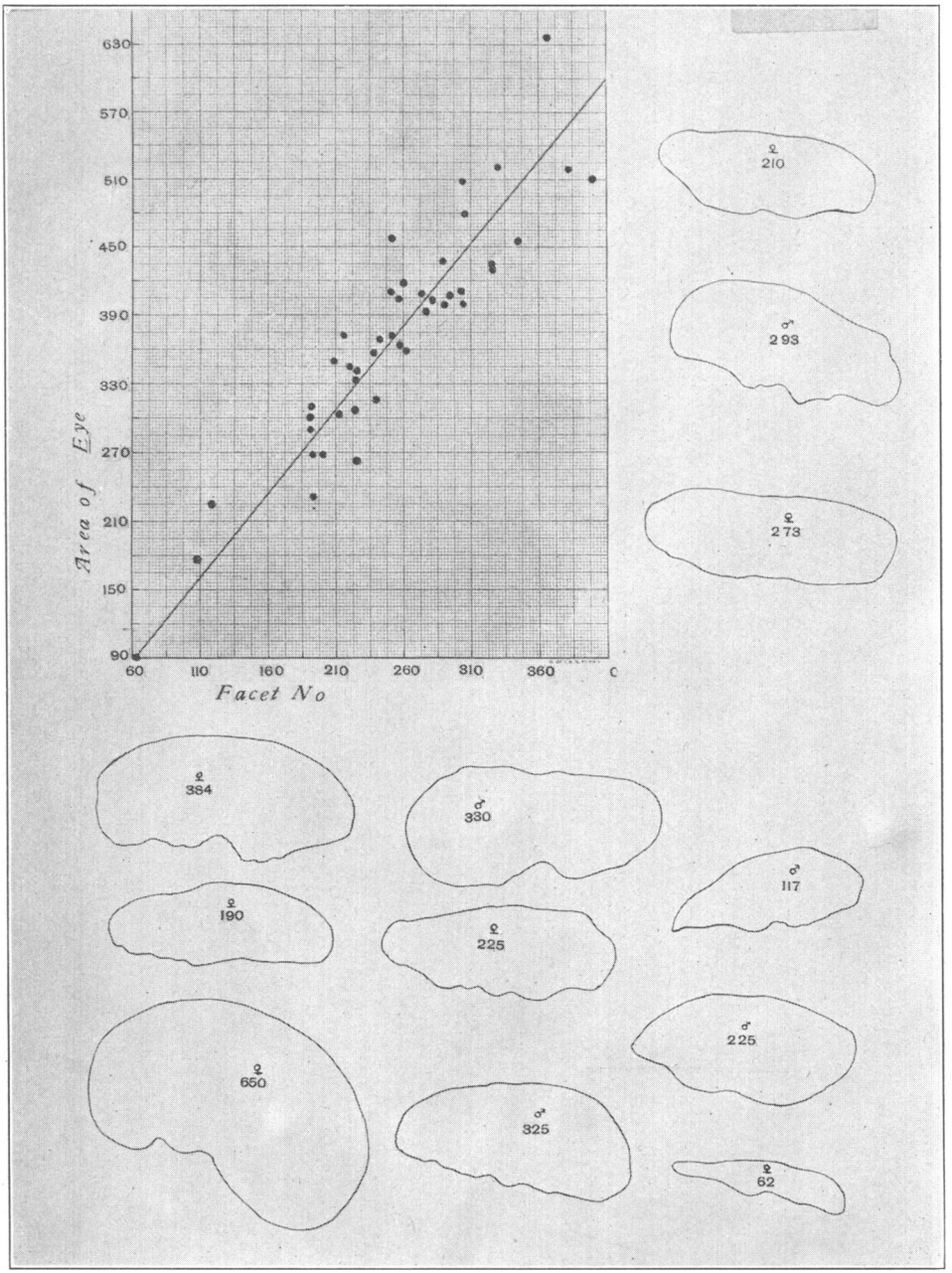
6. Temperature is effective only during the larval period.

ACKNOWLEDGMENT.

This work was done under the direction of Dr. Charles Zeleny and I wish to express my thanks to him for his interest in the problem and for his many suggestions while it was in progress.

EXPLANATION OF PLATE I.

Relation of facet number to area of eye in white bar-eye. Distribution of forty-three cases around average represented by line. Camera lucida drawings of eyes, with facet number.


630
570
510
450
390
330
270
210
150
90
60
110
160
210
260
310
360
Area of Eye
Facet No
♀ 210
♂ 293
♀ 273
♀ 384
♂ 330
♂ 117
♀ 190
♀ 225
♂ 225
♀ 650
♂ 325
♀ 62